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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/734,936	12/12/2003	Wonchul Suh	CL1878USNA	2510

23906 7590 12/20/2005

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EXAMINER	
MCGILLEM, LAURA L	
ART UNIT	**PAPER NUMBER**
1636	

DATE MAILED: 12/20/2005

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/734,936	SUH, WONCHUL	
	Examiner	Art Unit	
	Laura McGillem	1636	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *01 November 2005*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-30* is/are pending in the application.

4a) Of the above claim(s) *2,18-19* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1,3-17 and 20-30* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☒ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on *12 December 2003* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date *2/9/04, 1/26/05*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

Election/Restrictions

Applicant's election without traverse of Group I (claims 1, 3-17 and 20-30) in the reply filed on 11/1/2005 is acknowledged.

Claims 2, 18-19 are withdrawn from further consideration pursuant to 37 CFR 1.142(b) as being drawn to a nonelected invention, there being no allowable generic or linking claim. Election was made **without** traverse in the reply filed on 11/1/2005.

Claims 1, 3-17 and 20-30 are pending.

Specification

The use of the trademarks GENBANK (paragraph 0111, 0124, 0157, 0158, 0245 and 260), QIAGEN (paragraphs 0126, 0127 and 0262), AMPLI TAQ GOLD (paragraph 0262), QIAQUICK (paragraph 0263) and SORVALL (paragraph 0266) has been noted in this application. They should be capitalized wherever they appear and be accompanied by the generic terminology.

Although the use of trademarks is permissible in patent applications, the proprietary nature of the marks should be respected and every effort made to prevent their use in any manner which might adversely affect their validity as trademarks.

Claim Objections

Claim 3 is objected to because of the following informalities: the phrase "the first or second the expressible" is grammatically incorrect. It would be remedial to remove the word "the" between "second" and "expressible". Appropriate correction is required.

Claims 11 and 15 are objected to as being directed to claim 2, which is non-elected subject matter and which has been withdrawn by Applicant in the response filed 11/1/05.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 1, 3-17 and 20-30 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 1 step (a) (ii) and Claim 17 step (b) (ii) are vague and indefinite because they recite the phrase "RR3 is a third recombination of " and it is not clear how a structural element is a recombination. For purposes of examination, they will be interpreted as a "recombination region".

Claims 10 and 24 recite the limitation "the phage" and "the *lac* promoter". There is insufficient antecedent basis for this limitation in the claims. There is no prior mention of any of the claimed promoters.

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 1, 3-17 and 20-30 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for directed integration of a DNA fragment into an *E. coli* chromosome, does not reasonably provide enablement for directed integration of a DNA fragment into the chromosomes of any bacteria or any recombination proficient host cell. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to use the invention commensurate in scope with these claims.

The test of enablement is whether one skilled in the art could make and use the claimed invention from the disclosures in the application coupled with information known in the art without undue experimentation *United States v. Telectronics*, Inc., 8 USPQ2d 1217 (Fed. Cir. 1988). Whether undue experimentation is required is not based upon a single factor, but rather is a conclusion reached by weighing many factors. These factors were outlined in *Ex parte Forman*, 230 USPQ 546 (Bd. Pat. App. & Inter. 1986) and again in *In re Wands*, 8 USPQ2d 1400 (Fed. Cir. 1988) and include the following:

1) Scope of the claims The claims are drawn to a method for directed integration of an expressible DNA element into a bacterial chromosome of a bacteria or a recombination proficient host cell *in vivo* at a bacterial chromosome region homologous to regions of recombination elements. As written, the claims are drawn to all types of bacteria and recombination proficient host cells, which comprises an

extremely large group and includes well-known bacteria species with sequenced genomes, as well as bacteria with unsequenced genomes.

2) Nature of the invention. The invention involves molecular biology and chromosomal homologous recombination which is a complex, unpredictable aspect of science.

3) Working examples. Applicants have provided an example of directed integration of a *dxs* promoter into the *E.coli* chromosome, including exemplification of synthesis of PCR-generated DNA fragments with homology arms that match sequences in the *E.coli* chromosomal DNA, how to transform *E.coli* with linear DNA fragments, eliminate the selectable marker using site-specific recombinase reaction and confirm the integration of the promoter or foreign gene in front of a *dxs* or an *idi* gene on the *E.coli* chromosome. Applicant has also exemplified integration of genes from *Methylomonas* or *P. stewartii* into *E.coli*.

4) Amount of guidance provided. Applicant has provided guidance on how to direct integration of a small number of foreign promoters or expressible DNA fragments into an *E.coli* chromosome. Applicant has provided guidance on how to synthesize flanking "homology arms" to facilitate homologous recombination with particular *E.coli* chromosomal regions. Applicant has not provide guidance on how to perform directed integration of any type of expressible DNA fragment or foreign promoter into the chromosome of any other type of bacteria, including those bacteria for which the chromosome genomic sequence has not been determined. Applicant has not provided guidance on how to determine an effective or significant region of interest for

chromosomal integration into any bacteria other than *E.coli*. Applicant has not provided guidance on how to design nucleotide fragments that would be homologous to potential recombination sites in the chromosome of any type of bacteria, which would entail having some knowledge of the location of genes coding for proteins of interest, as well as sequences which flank the open reading frame of a protein of interest.

5) State of the art. A recent publication (Microbial Genomics Research, 2005) from the Department of Energy (DOE) describes progress of the Genomics:GTL program aimed towards sequencing the genomes of microorganisms with potential environmental, energy, health or industrial applications. The DO teaches that approximately 200 bacterial genomes have been sequenced or are in the process of being sequenced. Coenye et al (FEMS Microbiol Rev. 2005. Vol. 29 Pp.147-67) teach that microbial genome sequencing is very useful for determining microbial taxonomy, but that it is unlikely that genome sequences will become available for many species in the near future (see page 159, left column, last paragraph, in particular).

Yu et al (of record) teach a recombination (λRed) system for chromosome engineering in *E.coli* that is based on a λ prophage comprising three recombination genes (*exo, bet* and *gam*) under the control of a temperature sensitive regulatory gene. The ability of the host cell to become recombinogenic is reliant on a temporary increase in temperature to 42°C to activate the three recombination genes, *gam*, in particular (see page 5978, right column, 1st full paragraph in particular).

6) Unpredictability of the art. The unpredictability of being able to perform a method for direct integration of expressible DNA fragments into specific regions of

bacterial chromosomes stems from the sheer amount of bacterial species or recombination proficient host cells that are known and the genomic variability among these many species, even among those relatively few bacteria or host cells with sequenced genomes. Coenye et al (FEMS Microbiol Rev. 2005. Vol. 29 Pp.147-67) teach that genomic sequence of even one strain of *E.coli* is not sufficient to represent the diversity of a species and the gene content of various *E.coli* strain genomes has been found to be as different as 29.2% (see page 148, right column, 1st full paragraph, for example). Coenye et al also teach that multiple factors are commonly responsible for altering bacterial genomes including gene duplication, horizontal gene transfer, gene loss and chromosomal rearrangements (see page 149, left column 1st paragraph, for example) and suggests that genomes of even related bacterial species may be very different. For example, Coenye et al teach that comparison of the genome of *Mycobacterium tuberculosis* with its relative *Mb. leprae* reveals that *Mb. Leprae* has 2000 fewer genes through gene loss (see page 151, left column, 1st paragraph). Coenye et al discloses that the microbial organisms which have been sequenced to date are "by no means representative for total prokaryotic diversity". Coenye et al further observe that although the organisms which have been sequenced are largely important for medicine and biotechnology, more than 99% of all naturally occurring microorganisms cannot be cultured using standard techniques (see page 161, right column, 2nd full paragraph, in particular).

Furthermore, the ability of any type of bacterial cell other than *E.coli* to be " a recombination proficient bacterial host" is unpredictable. The specification defines

"recombination proficient bacterial host" as a bacterial host that contains a functional recombination system and is capable of homologous recombination at rates useful for genetic engineering (see paragraph 0176). The λRed recombination system is advantageous to use in an *E.coli* host because the *exo, bet* and *gam* gene are useful to inhibit endogenous intracellular exonuclease activity that degrades linear DNA and inhibits transformation; however, since this recombination system is reliant on a specific temperature change to activate expression of the crucial recombination system, it is unpredictable whether this particular recombination system would be functional in any other bacteria other than *E.coli* especially for bacteria that might be less tolerant of said temperature change in order to be recombination proficient or capable of homologous recombination at rates "useful for genetic engineering."

Given the amount of diversity that is present in the genomes of species that are known, the potential for genomic alteration by multiple pathways, and numbers of known bacterial species, the ability to integrate an expressible DNA or a foreign promoter into any type of bacterial chromosome or any type of recombination proficient host cell using homologous chromosomal regions is unpredictable. One of skill in the art would have to practice trial and error experimentation in order to produce and use claimed recombination elements to directly integrate DNA into any chromosomal region of any bacteria or recombination proficient host cell in order to perform the claimed method.

Given the above analysis of the factors which the Courts have determined are critical in ascertaining whether a claimed invention is enabled, it must be considered

that the skilled artisan would have had to have practiced undue and excessive experimentation in order to practice the claimed invention.

Conclusion

No claims are allowed.

The closest prior art to the instant inventive method includes Perkins and Tugendreich (U.S. Patent Application Publication No. US 2002/0151058), who teach recombination methods using intermediate expression vectors containing two sequence specific recombination regions (i.e. triple homologous recombination, see Figure 3). However, Perkins and Tugendreich do not teach the use of the λRed recombination system, recombination into a bacterial chromosome or a second recombinase reaction to eliminate the selectable marker. In addition, Zhang et al (of record) teach site-specific recombination for chromosomal engineering in *E.coli* (see page 125, Figure 3, for example), but do not teach the use of the λRed recombination system, or triple homologous recombination.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Laura McGillem whose telephone number is (571) 272-8783. The examiner can normally be reached on M-F 8:00-5:00.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Irem Yucel can be reached on (571) 272-0781. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

Laura McGillem, PhD
12/12/2005

DAVID GUZO
PRIMARY EXAMINER